May

Week 1 (02/05/16 - 06/05/16)

- MODULE 1:

Competent cells protocol was performed in order to carry out a transformation protocol for biobrick BBa_J04450.

- MODULE 2:

Lyophilized plasmid of BBa_J04450 was resuspended in order to be transformed into competent cells previously prepared.

- MODULE 3:

SOC medium was prepared, after one day it kept its clean color, so it will be used later in the transformation protocol.

- MODULE 4:

Transformation protocol was carried out and the resultant cells were cultured in a stria way into an agar+LB+Cloramphenicol (35 μg/ml) Petri dish plus an agar+LB Petri dish as a negative control. A method in which the Petri dishes culture and tube culture are performed within one unified protocol was made.

Summary

Each protocol was successfully carried out, except for the BBa_J04450 transformation, since no growth occurred in the agar+LB+Cloramphenicol(35 μg/ml) Petri dish although growth could be seen in the control dish, which indicated the success of the competent cells fabrication, however the transformation protocol failure was also demonstrated. Further transformation protocols must be performed in order to proceed with the characterization process of Bba_J04450.

Week 2 (09/05/16 - 13/05/16)

- MODULE 1:

The transformation product was cultured within Agar+LB+Cloramphenicol (35 μg/ml) Petri dishes and an Agar+LB Petri dish. Colonies growth was confirmed.

- MODULE 3:

After colony growth was confirmed in both the control and the Petri dishes + antibiotic, stria culture was executed taking 5 different colonies to be recultured in 5 stria. The stria culture didn't show any growth.

Summary

Further stria culture must be performed from 5 different colonies to perform tube and flask inoculation, plasmid extraction and further protocols possible.

Week 3 (16/05/16 - 20/05/16)

- MODULE 1:

Agar+LB+Chloramphenicol (35 μg/ml) and Agar+LB Petri dishes were prepared in order to seed the transformed E-coli DH5-alpha cells.

- MODULE 2:

5 colonies were taken from the transformation product referenced during week 2 , on module 1.
Stria of each colony was cultured in agar+LB+Chloramphenicol (35 μg/ml) petri dish.
3 of the 5 stria showed consistent growth within an overnight time lapse incubation.

- MODULE 3:

2 stria were inoculated into 15 mL LB tubes, which were incubated at 37ºC and 240 rpm. Neither of them tubes showed growth.

- MODULE 4:

The remaining stria from the ones grown in Module 2 were cultured in a stria way into an agar+LB +Chloramphenicol (35 μg/ml). No growth was reported.

Summary

Since the stria didn't show any growth and since we need to re-seed them into LB tubes and flasks to eventually proceed with a mini-prep plasmid extraction, further tries must be carried on to obtain the necessary growth for characterization of Bba_J04450.

Week 4 (23/05/16 - 27/05/16)

- MODULE 1:

5 colonies were taken from the previously seeded E-coli DH5-alpha cells transformed with Bba_J04450 and cultured in a stria way in an agar+LB+chloramphenicol(35 μg/ml) Petri dish. 4 of the 5 strias grew successfully.

- MODULE 2:

2 strias were re-seeded into 15 mL tubes and were incubated overnight at 37ºC and 240rpm. Both tubes showed growth.

- MODULE 3:

The grown cells within the tube were taken and re-seeded into a 150mL flask, which were incubated overnight at 37ºC and 240rpm. The flask showed successful growth.

- MODULE 4:

Miniprep plasmid extraction was carried out with the transformed bacteria previously transformed and re-seeded into a 150mL LB flask. The resultant product was stored at -20ºC for subsequent procedures to be done.